FAUNAL SURVEY. II. THE DISTRIBUTION OF DIGENEAN TREMATODES WITHIN THE NEW ENGLAND TABLELANDS

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Trematode larval stages collected from aquatic snails across the New England Tablelands were examined and identified through morphometric analysis and where possible, transmission experiments and 28S (LSU) rDNA analysis. One monostome and three distome digenean trematode species were found in populations of *Gabbia vertiginosa* (Bithyniidae). One distome digenean was found in the snail *Glyptophysa* sp. (Planorbidae). The prevalence of all trematode species was low and their distributions limited to the central portion of the New England Tablelands. Temporal and spatial heterogeneity within both intermediate and definitive host populations may be acting as isolating mechanisms at times of recruitment of larval trematodes, limiting parasite distributional ranges through disruption of their natural transmission cycles. ☐ *Trematode, Notocotylidae, Echinostomatidae, Heterophyidae, distribution.*

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Aquatic snails collected during a survey of the New England Tablelands, New South Wales, between February 2000 and 2003 (Koch, 2004) were examined for larval stages of trematodes. Typically, such parasites utilise molluscs as intermediate hosts in two or three-host life cycles (Ginetsinskaya, 1988). Sporocyst, redia, cercaria and metacercaria stages of trematode development can be found in both terrestrial and aquatic snails, with adult parasites maturing within a definitive vertebrate host (Rohde, 2001; Haas & Haberl, 1997). Traditionally trematodes have been identified to genus and species level by obtaining a mature adult worm either by dissection out of a natural vertebrate host or by completing life cycle experiments utilising the larval forms obtained from snails collected from a natural habitat (Gibson, 1998; Wright, 1971). Whenever possible, such transmission experiments were attempted throughout this survey period. In addition to the traditional identification methods, partial sequencing of the 28S long subunit (LSU) rDNA taken from larval trematodes found during dissections of snails was undertaken at the Natural History Museum, London), (Olson et al, 2003; Littlewood & Olson, 2001).

The aims of the survey were to identify trematode species across the New England Tablelands; to establish their distributional ranges; and to identify the aquatic snails being parasitised, and thereby determine intermediate host specificity.

METHODS

The Australian Biogeographical Integrated Grid System (ABIGS) was used to map all species, in accordance with Brook (1977) and Simpson & Stanisic (1986). Aquatic snails were collected as described by Koch (2004). Snails were dissected within 24 hours of collection by crushing of the shell and the tissue examined in a cavity block under a dissecting microscope for live trematodes. Trematode specimens were fixed in either 10% hot formalin or hot saline (8.5g NaCl per 1 litre distilled water), stained with either Grenacher's carmine alum or acetocarmine, dehydrated in an alcohol series, and mounted in Canada balsam. Slides were then dried in an oven at 66°C for 14 days (Conn, 1946). Specimens required for DNA sequencing were dropped immediately upon dissection into absolute alcohol (Jousson et al., 1998). Identification of trematodes was done primarily by obtaining adult worms through life cycle experiments whenever possible, and secondarily through partial sequencing of rDNA 28S LSU by Olson and Littlewood. In all other cases, cercarial and redial life stages were used to identify species to family and/or genus using published research papers and keys (Yamaguti, 1971, 1975; McDonald, 1981). Intensity was defined as the number of cercariae observed within the snail tissues at the time of dissection (high: >100 cercariae per snail; medium: 50-100 cercariae per snail; low: <50 cercariae per snail). Prevalence was defined as
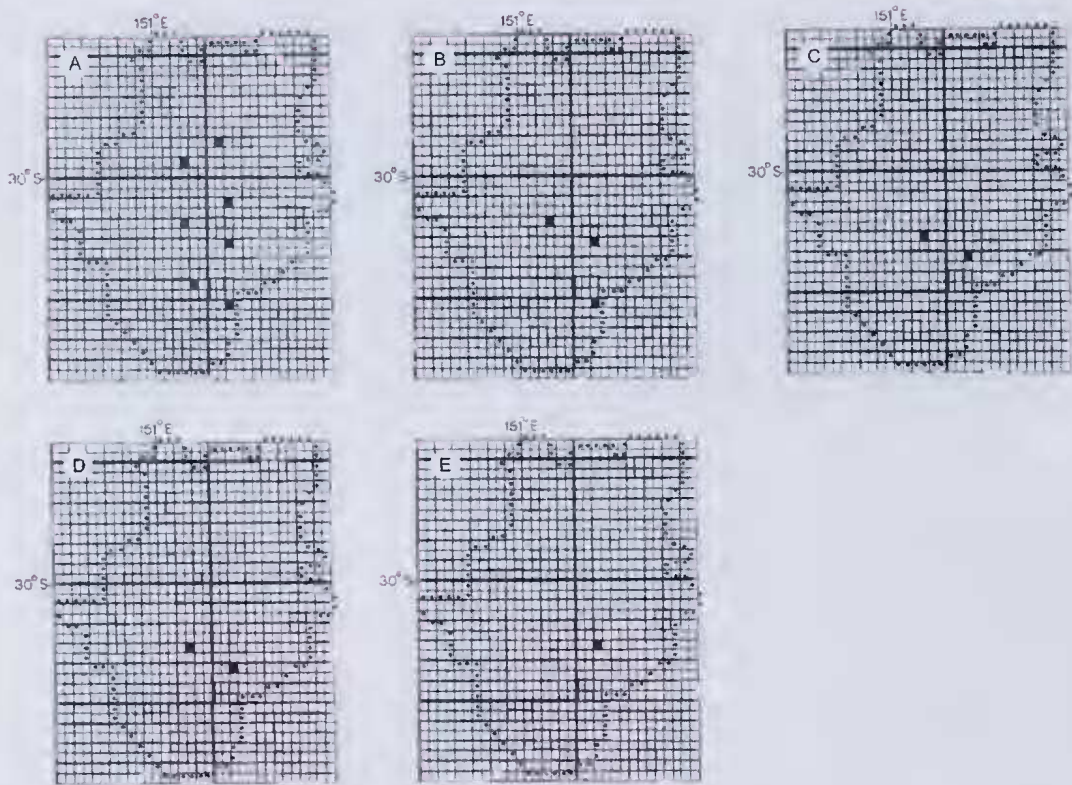
FIG. 1. Comparison of current distributional ranges of trematodes with that of their intermediate snail host, *Gabbia vertiginosa*. A, *G. vertiginosa* ; B, *Catatropis indicus*; C, *Philopthalmus* sp. nov.; D, *Echinoparyphium australis* n. sp.; E, *Ascocotyle* sp. nov. (ABIGS system grid superimposed over the New England region with the surveyed area enclosed by the solid dots — see Koch, 2004)

the percentage of snails collected that were infected by trematode larval stages.

Photographs were taken using an Olympus BH2 camera attached to a stereomicroscope, or using a digital camera attached to a Nomarski contrast microscope. Digital photographs were downloaded into Adobe Photoshop program for editing. Body parts were measured with a calibrated eyepiece graticule. Measurements are given in millimetres.

RESULTS

Two snail species were found to be parasitised. The prosobranch *Gabbia vertiginosa* (Bithyniidae) was host to 1 monostome and 3 distome digeneans: *Catatropis indicus* (Notocotylidae), *Echinoparyphium australis* sp. nov. (Echinostomatidae), *Philopthalmus* sp. nov. (Echinostomatidae), and *Ascocotyle* sp. nov. (Hetcrophyidae) (Figs 1-3). Adult worms of *C. indicus* and *Echinoparyphium australis* sp. nov. were obtained from life cycle experiments (Koch, 2003). Phylogenetic analysis

of the LSU 28S rDNA of adult worms against 150 digenean taxa confirmed the identity of *Echinoparyphium australis* sp. nov. (Genebank accession number AY395577) and *C. indicus* (Genebank accession numbers AY222114, AY222220). PCR products could not be generated for any other trematode species. Attempts to obtain adults of *Philopthalmus* sp. nov. and *Ascocotyle* sp. nov. failed.

The rediae and cercariae of *C. indicus* always occurred alone within the gonad tissue of *G. vertiginosa*. However, the other three trematode species were found as concurrent interspecific double and triple infections within *G. vertiginosa*, all utilising gonad and digestive gland tissues. *Philopthalmus* sp. nov. established infection first within the snail. Recruitment of *Echinoparyphium australis* sp. nov. and *Ascocotyle* sp. nov. followed in that order.

Glyptophysa sp. (Planorbidae) was host to one distome digenean, *Echinostoma* sp. nov.
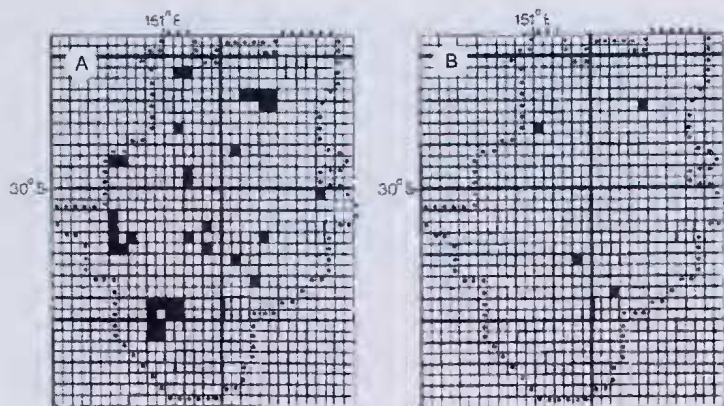
FIG. 2. Comparison of current distributional ranges of *Echinostoma* sp. nov. with that of its intermediate snail host, *Glyptophysa* sp.

(Echinostomatidae) with rediae and cercariae occurring in digestive gland and gonad tissue of the snail (Figs 2 & 3E). It was present in the snail population during all seasons, across all years. Adult worms of this species could not be obtained through transmission experiments.

C. indicus was present in the snail populations at all seasons across all collection years. The three distome species specific to *G. vertiginosa*, however, were present only between January and April of all collection years. Intermediate host specificity was high for all trematode species as demonstrated by the use of a single host species and failure of parasites to establish infections in other aquatic snail species during transmission experiments performed and described by Koch (2003). Prevalence and abundance of all trematode species are summarised in Table 1.

Detailed descriptions of parasite anatomy and life cycles, and transmission experiments can be found in Koch (2002, 2003).

DISCUSSION

Many studies have presented data to support the hypothesis that temporal heterogeneity within both definitive and intermediate host populations can bring about the isolation and even removal of a parasite species from the environment when the chronological patterns of activity of the hosts are in opposition either with the parasite or each other (Al-Kandari et al, 2000; Kuris & Lafferty, 1994; Crews & Esch, 1986; Cort et al, 1960). The trematode life cycle has evolved around this variation in both intermediate and definitive host habitat usage, and the interplay of temporal behaviour patterns of both host and parasite (Poulin, 1999a; 1999b; Poulin &

Morand, 1999). Seasonal migration and other movements across and between habitats by final hosts such birds can remove or add hosts from or to the specific preferences of parasite species. By adding to the habitat, the opportunity for completion of a life cycle in one or more final hosts by one or more parasite species is greatly enhanced. Conversely, the departure of a final host from a particular habitat may cause the loss (seasonally or permanently) of one or more parasite species due to the interruption of the natural cycle of transmission between intermediate and definitive hosts (Combes et al, 1994; Kuris & Lafferty, 1994). Such temporal pulses in host populations will bring about pulses in parasite populations actively associated with those hosts (Smith, 2001). An inference can be drawn from my study of a degree of temporal variation in both parasite and intermediate host populations between seasons as indicated by the presence of distome species in *G. vertiginosa* only in mid and late summer months. Published records show natural final hosts of the Echinostomatidae and Heterophyidae to be commonly waterbirds such as ducks, herons, and egrets (Kanev et al, 1998; Yamaguti, 1975). These species are mostly nomadic birds that are found abundantly across the Tablelands during the summer months, moving towards the warmer coastal or inland areas for winter. Such migration patterns can link habitats and thus parasite communities of intermediate hosts creating pulses in prevalence in both parasite and intermediate host populations across time (Smith, 2001). There was a consistency in my study, however, of the presence of all parasite species across years, indicating only seasonal isolation of species within habitats as opposed to complete removal of species.

Spatial heterogeneity in parasite recruitment due to geographical isolation of snail host populations and possible definitive host habitat preferences may be responsible for the patterns and seasonality of parasite distribution along the central section of the Tablelands. This area has a high concentration of natural watercourses that attract water bird species and provide suitable habitats for first and second intermediate hosts
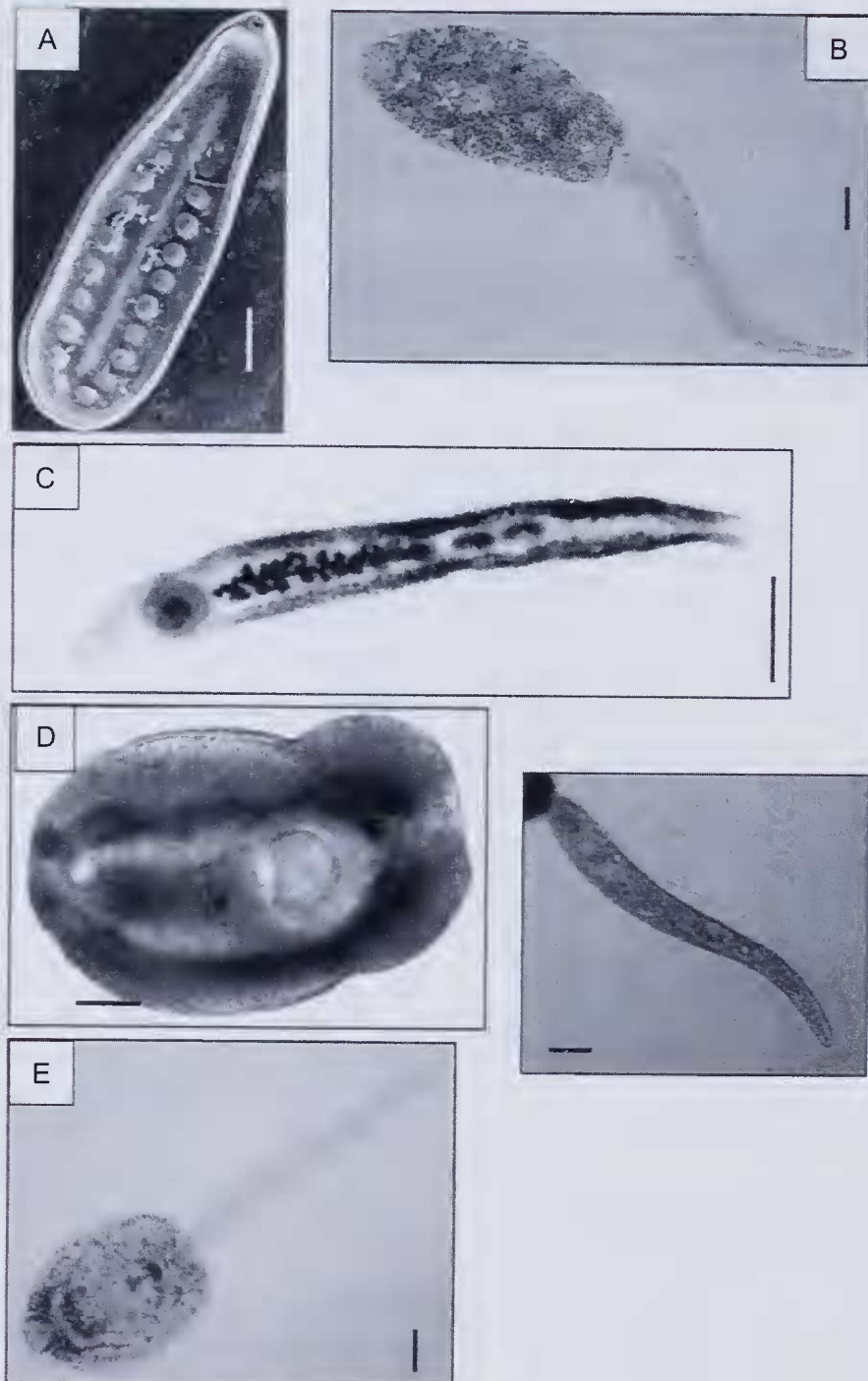
FIG. 3. Trematode species found during the survey of aquatic gastropods of the New England region. A, *Catatropis indicus* adult (SEM); B, *Philopthalmus* sp. nov. Cercaria; C, *Echinoparyphium australis* sp. nov. adult; D, *Ascocotyle* sp. nov. cercaria with close-up of tail finfold; E, *Echinostoma* sp. nov. cercaria. Scale bars: A & C = 1mm; B,D,E = 20μm.

TABLE 1. Prevalences of infection of larval trematodes within aquatic snail populations of the New England Tablelands.

Parasite	Prevalence	Intensity	Snail Host
Catatropis indicus	3.5 % (5240)	high	*Gabbia vertiginosa*
Philopthalmus sp. nov.	0.90 % (5240)	high	*Gabbia vertiginosa*
Echinoparyphium australis sp. nov.	0.30 % (5240)	medium	*Gabbia vertiginosa*
Ascocotyle sp. nov.	0.08 % (5240)	low	*Gabbia vertiginosa*
Echinostoma sp. nov.	85% (47)	high	*Glyptophysa* sp.

such as snails, fish and frogs (Douglas & Douglas, 1977). There are also many man-made dams and swamps, some of which do not support the diversity of invertebrate and vertebrate species required for completion of digenean trematode life cycles (Jacobs, 1952; pers. obs.). A large proportion of these habitats dry up for three to five months every year, concentrating snails and final hosts within a small distribution range. These factors may act as potential isolating mechanisms occurring at times of recruitment of parasites and peak snail host reproductive periods, in the summer months of October through to April. All trematode species in the study demonstrated high inter-mediate host specificity (Koch, 2002, 2003), a trend shown to be common particularly within the Echinostomatidae (Sapp & Loker, 2000; Korner et al, 1995). When host species demonstrate such uniquely specific assemblages of trematode species, isolation of these species over both space and time, and thus their recruitment into snail host populations, is more likely to occur than in those parasite species with low host specificity (Kuris & Lafferty, 1994).

The impact of larval stages such as rediae and cercariae on intermediate host fertility and reproduction could cause substantial host population reductions if prevalences were as high as those described in other studies (Huxham et al., 1993; Kuris, 1973; Sousa, 1993; Irwin, 1983; Pan, 1965). However, the high abundances of larval stages combined with low prevalences in the New England region provide for an environmental system within which sustainability of both host and parasite populations is possible.

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